Project Title:	The Virtual Liver Project
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Key NCCT Participants:	Jason Pirone, Postdoctoral Fellow (TBN), Rory Conolly, Tom Knudsen, Hugh Barton, Richard Judson, David Dix, Keith Houck, Woody Setzer, Jerry Blancato, Robert Kavlock

1. Short Description of Project

The objective of the Virtual Liver is to aid in assessing the human risk of chronic liver toxicity *in silico* from environmental chemicals. We believe computational systems modelling can be used predict organ injury due to chronic chemical exposure by simulating: (i) the dynamics of perturbed molecular pathways, (ii) their linkage with adaptive or adverse processes leading to alterations of cell state, and (iii) the integration of the molecular and cellular responses into a physiological tissue model. This will be accomplished through two strategic initiatives: (a) a knowledgebase (KB) to logically model the relevant physiologic entities and their interactions at molecular, cellular and tissue scales; and (b) a multiscale agent-based simulation to predict the dose-dependent perturbations of pathways to chronic liver injury. The 1-2 year goal of the project is to focus on modeling a physiologic outcome (e.g. hyperplasia) as a proof of concept. In the next 3-5 years this will be expanded to evaluate an apical toxicity endpoint across rodents and humans. The long-term vision for the project is to provide estimates for the risk of injury due to different chemicals, across genders, life-stages and populations. If successful, the Virtual Liver is expected to reduce dependence on animal testing through effective *in silico* predictions.

2. What is the EPA Context for this Project ?

Assessing the quantitative risk of human injury by extrapolation from animal toxicity studies is fraught with uncertainty because: (a) pathways of chemical-induced injury can vary between species; and (b) pathways activated by high-doses of chemicals may not be the same as those stimulated by low-dose long-term exposure. The increasing scientific, ethical and economic impetus for alternative toxicity testing approaches was highlighted in a recent report (NRC, 2007). In addition to enabling more scientifically based estimates of risk for human toxicity, it is also vital to predict the level of risk based on age, gender or other susceptibility criteria. Currently, the EPA ToxCast™ program is evaluating molecular and cellular in vitro assays to empirically predict long-term toxicity for chemical prioritization (Dix et. al., 2007). These data provide a valuable opportunity, in conjunction with other data streams, for quantitative modeling using *in vitro* data.

Animal studies show a range of chemical-induced injury including developmental defects, reproductive effects, neurological dysfunction, and organ damage (liver, kidney, lung, etc). The *critical effect*, which is the first adverse effect observed with an increasing dose of the test chemical, is used to estimate the allowed exposure level of a chemical. Figure 1 shows the distribution of critical effects by organ for more than 500 orally consumed environmental chemicals from the US EPA Integrated Risk Assessment System (IRIS) www.epa.gov/iris. <u>The liver is the most frequent source of critical effects</u>. As the primary organ for metabolising chemicals in the body, the liver is the most common and earliest site of injury for a wide range of environmental toxicants. Therefore understanding and predicting dose-dependent

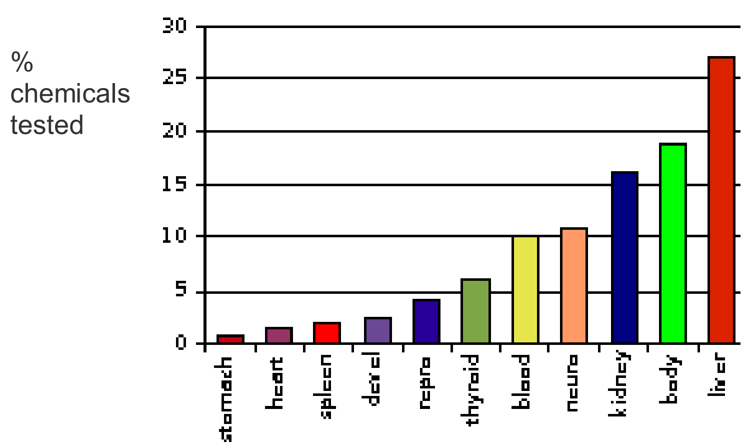

Figure 1. Percentage of chemicals tested showing critical effects by organ

liver injury should have a broad impact on assessing the risk of new chemicals.

Figure 2. Shows some of the key events in the modes of liver injury. The acute or chronic nature of chemical exposure has been found to play a role in determining the type of liver damage. The mode of action for acute injury often includes cytoplasmic, mitochondrial and nuclear alterations (e.g. hydropic, fatty, Mallory bodies, etc) that can lead to necrosis. For chronic liver injury, one mode of action includes recurring damage, e.g. necrosis followed by regenerative proliferation, which causes fibrosis (scarring) that may lead to cirrhosis. Chronic exposure can also cause cancer through at least two major modes of action: genotoxic and non-genotoxic. Genotoxic cancer occurs in multiple steps: (i) initiation, in which chemical mutagens cause DNA damage rendering a cell resistant to apoptosis, inhibition of cell proliferation, and (ii) promotion, in which mitogenic signals persistently stimulate the initiated cell creating focal proliferation. This can lead to neoplastic legions. In non-genotoxic cancer, persistent molecular perturbations due to xenobiotics are believe to deregulate cell cycle checkpoints, increasing cell proliferation, which in turn elevates the risk of genotoxic neoplastic lesions. Critical effects in the liver are often graded by propensity for cancer: non-neoplastic (e.g. necrosis, inflammation, steatosis, fibrosis), preneoplastic (e.g. proliferation, hyperplasia), and neoplastic (e.g. adenoma, carcinoma). Animal testing points to liver cancer as one of the important chronic liver toxicity endpoints for environmental chemicals (Martin, *et. al.* 2007). While mutagens can be identified readily through DNA damage assays ruling out genotoxic cancer, non-genotoxic carcinogens have no established biomarkers and present a serious challenge for risk assessment. One mode of action for non-genotoxic cancer begins with nuclear receptor (NR) activation. Surprisingly, a number of high volume environmental chemicals including, pesticides (conazoles and pyrethroids), flame retardants (DE-71), plasticizers (phthalates) and other persistent toxic substances (PFAAs, PCBs) are non-genotoxic rodent carcinogens and nuclear receptor (NR) activators. Thus, a focus on modeling NR-mediated non-genotoxic cancer by (i) mechanistically relating early molecular events to cell perturbations, cellular perturbations to tissue lesions; and (ii) extrapolating these mechanisms between species, will provide valuable tools for assessing the risk of key environmental chemicals of relevance to the EPA.

Molecular Perturbations ➡	**Cellular Perturbations** ➡	**Tissue Injury**
•Nuclear receptor signaling •Enyzme induction •Oxidative Stress •Xenobiotic metabolism •Endogenous metabolism •MAPK signaling •Caspase activation •Inflammation •Cell Cycle •DNA Damage	•Hypertrophy •Alteration •Proliferation •Initiation/ promotion •Death -Apoptosis -Necrosis	•Non-neoplastic -Necrosis -Fibrosis -Cirrhosis -Choleastasis -Granuloma •Neoplastic -Adenoma -Carcinoma

Figure 2. Key events in liver injury

3. What are The Strategic Directions and Scientific Challenges ?

Our hypothesis is that chronic liver injury is due to: (a) the response of molecular networks perturbed by xenobiotics, (b) the dynamics of adaptive cellular processes coping with external stress leading to the preservation or alteration of cell state (survival/division/death), and (c) the tissue dynamics as networks of cells adapt in response to gradients of xenobiotics and nutrients carried by blood flow giving rise to normal or adverse histomorphologic changes. Testing this hypothesis *in silico* presents two important computational challenges. First, qualitative insight into mechanisms is incomplete and dispersed across disparate structured or unstructured biological information sources. Second, modeling tissue injury dynamics in terms of complex molecular, cellular and tissue processes is difficult. We propose two strategic directions to address these challenges: (i) a knowledgebased approach to organize complex mechanistic information, and (ii) a multiscale simulation approach to analyze histomorphometric changes emerging for the collective behavior of liver cells, where each cell autonomously processes local information through programmes encoded in molecular circuits. These strategic initiatives are aligned with the National Research Council's report on "Toxicity Testing in the 21st Century: A Vision and a Strategy," which emphasizes the need for innovative approaches for testing toxicity of chemicals in humans.

Knowledgebased, or semantic, approaches (Karp 2001) deal with the incomplete and evolving insight on complex mechanisms. They enable integration of disparate biological information from literature, -*omic* data, or pathway databases at different scales into coherent mechanistic representations in a flexible, extensible, transparent and logical framework. The Liver Knowledgebase (KB) will manage mechanistic concepts formally (through ontologies) and associated facts that will be amenable to computation, queries and visualization for a range of scientists and risk-assessors across the EPA. Large-scale knowledgebased approaches have become feasible due to semantic web technology and ontologies are being developed to represent different levels of biological organization. The recently established National Center for Biomedical Ontology[1] provides a resource for the broader application of knowledgebased approaches in biology and will be leveraged by the proposed effort.

Virtual tissues are an emerging area of computational research aimed at integrating biological phenomena at multiple scales in order to simulate macroscopic behavior from cellular properties. Quantitative liver histopathology data has been used for modeling toxicity in population-based multistage models of carcinogenesis (Armitage 1954, Moolgavkar 1978) and for quantitative modeling of dose-dependent toxicity of environmental chemicals (Conolly 1993). Traditionally, physiologic modeling is used to study target-organ dose (PB/PK) and toxicity (PB/PD). More recently, computational physiology (Hunter 2003) has emerged as the integration of systems biology with physiology to model mechanisms at the molecular, cellular and tissue level. Virtual tissues are multiscale models that aim to predict behavior using dynamic models of molecular pathways, tissue structure and biofluid flow. Multiscale organ models have been developed for the heart (Nickerson et. al. 2005; Laganà et. al. 2005; Bassingthwaighte et. al. 2005; Shim et. al. 2006; Kerckhoffs et. al. 2007), the lung (Ma et. al. 2006; Klinke et. al. 2007), the brain (Robinson et. al. 2005) and tumour growth (Athale et. al. 2005). A multiscale model of hepatic function has not been developed thus far, though efforts on cellular models of hepatocytes are on-going under the auspices of the European Community HepatoSys project (Klamt et. al. 2007; Saez-Rodriguez et. al. 2006).

4. What are the short-term (1-2 year) and long-term (3-5 year) goals?

The 5-year plan for the Virtual Liver Project is to develop a knowledgebase for qualitatively describing species-specific toxicity pathways due to chronic exposure to chemicals, and to develop a virtual liver tissue that lays the foundation for quantitatively predicting the risk of non-genotoxic neoplastic lesions due to NR activators in humans. If successful the system will be extended to represent additional modes of action. The short-term goals of the project are to: (a) curate mechanistic knowledge about non-genotoxic cancer in rodents and humans and to formally describe the species differences; (b) develop initial dynamic models of literature-derived NR-mediated molecular interaction networks, beginning with xenobiotic metabolism; and (c) develop an initial tissue level model of the hepatic lobule representing the key cell types, their interactions, and the nutrients and xenobiotic gradient due to blood flow. The long-term goals are to: (a) extend the KB with additional molecular mechanisms; (b) expand the molecular interaction network to include additional key modules in relation to cell states; and (c) integrate the cell state model into the tissue model for multiscale simulation of injury.

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[1] http://www.bioontology.org

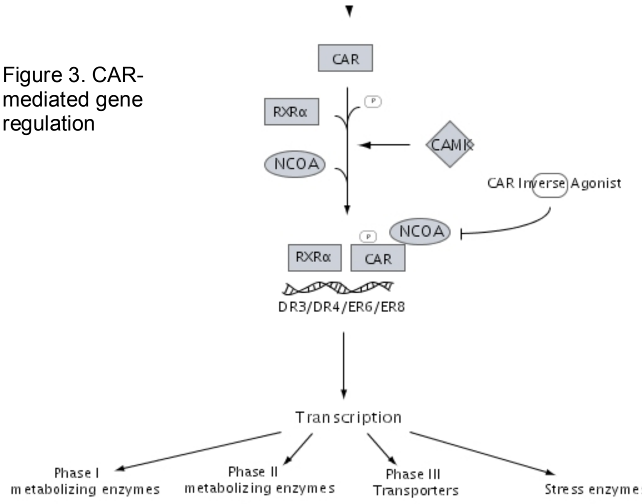

Figure 3. CAR-mediated gene regulation

Molecular and cellular scope. NRs activated by xenobiotics mediate the expression of enzymes that metabolize them to more soluble forms for excretion. Through pathways that are not completely understood, cell-cycle checkpoints can be deregulated, leading to increases in cell division. Chronic exposure to chemicals can lead to persistent cell proliferation, which can ultimately lead to the formation of neoplastic lesions. NRs are a multi-domain protein superfamily with ligand-binding (receptor) and DNA-binding (transcription factor) functions. The constitutive androstane receptor (CAR), pregnane X receptor (PXR), and peroxisome proliferator activated receptor alpha (PPAR-α) are NRs that transcriptionally regulate homeostatic responses to exogenous chemicals, for example xenobiotic metabolism. For example, tricholoroethylene (TCE) , di(2-ethylhexyl)-phthalate (DEHP) and perfluorooctanoic acid (PFOA) are PPAR-α activators (Maloney and Waxman 1999); while the pesticides methoxychlor, endosulfan, dieldrin, DDT, conazoles, and the plasticizer nonylphenol activate either PXR or both PXR and CAR (Kretschmer and Baldwin 2005). Increasing evidence suggests that NRs may mediate the toxicity of a number of environmental chemicals (Butler 1996). Some components of xenobiotic metablism have been well studied for CAR (Figure 3), PXR and PPAR-α, but many downstream intracellular and intercellular mechanisms leading to adverse outcomes remain to be deciphered. The genes, proteins and small molecules associated with the NR-mediated signaling and XME induction forms the first level of mechanistic information that will be curated in the knowledgebase. The second level of information will involve the organization of key intra-cellular processes leading to alterations in cell state (death/division). A great deal of information about NR-activators has been published. Furthermore, we are also designing rodent and human *in vitro* studies (in close collaboration with the ToxCast™ project) that will generate useful data key biological processes, transcript and metabolite profiles for liver cell types. These data will be used to verify curated knowledge, to discover causal molecular networks (D'haeseleer *et. al.* 2000), and to calibrate/evaluate a dynamic model of NR-mediated intracellular processes.

Tissue scope. The mammalian liver consists of around 10^5 to 10^6 lobules that receive blood supply from the hepatic artery and the portal vein. The hepatic lobule (Figure 4) is believed to represent the functional unit of the liver (Teutsch 2005) and will be used as the appropriate level for abstracting tissue-level response. Within each lobule liver cells are arranged in collateral plates one to two cells thick organized radially around the central vein. Blood flows from the hepatic venule and the hepatic arteriole though intervening spaces between the cells, called sinusoids (Motta 1974), and collects in the central vein. Regions of the lobule are divided into zones, based on oxygen gradient: zone 1 lies around portal tracts with the highest oxygen supply, zone 3 is surrounds the central vein and has the poorest oxygen supply, and zone 2 lies in between. The zones exhibit differential distribution of gene induction (Pette and Wimmer 1979; Oinonen *et. al.* 1998) proteins and metabolites. Furthermore, many lesions are also zonally distributed (Kato *et. al.* 2001). The cause zonation is not completely known but it has been attributed to development (Schmucker *et. al.* 1976; Lamers *et. al.* 1987), and also to the distribution of nutrients and oxygen across the lobule (Kietzmann *et. al.* 2006). The spatial organization of the lobule in terms of the cells and the blood flow are important is for understanding emergence of tissue-level injury due to

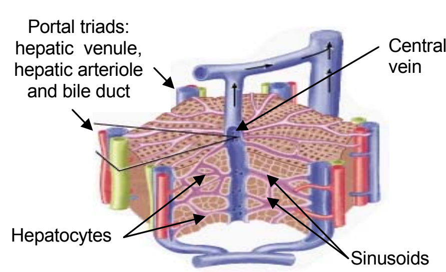

Figure 4. Hepatic Lobule

chemicals. Each lobule is composed of parenchymal cells (hepatocytes) and non-parenchymal cells, which occupy a much smaller volume but account for nearly half of the cell number. Non-parenchymal cells are believed to serve critical hepatic functions. For example, sinusoidal epithelial cells (SECs) are important for filtration, Kupffer cells (KC) are the resident macrophages that secrete mediators of inflammatory response, and hepatic stellate cells (HEC), also known as Ito cells, store fat, vitamin A and play a role in extracellular matrix remodeling (Kmieć 2001). Another cell type found in the sinusoids are granular lymphocytes, or Pit cells, that spontaneously kill different tumour cells. There is also increasing evidence that communication between parenchymal and non-parenchymal cells is relevant in chronic liver injury (Figure 5). KC have been shown to mediate liver toxicity and cancer for PPAR-α activators (Rusyn 1998; Roberts 2007); HEC are involved in fibrosis and fatty liver disease (Reeves 1996); and tumour-promoting agents disrupt gap junction intercellular communication (Krutovskikh et. al. 1995). The lobular entities including, the different cell types, sinusoids, blood flow and their relationships represent the tissue level information that will be curated in the KB. This information will be used to develop a spatial model of cellular networks in the hepatic lobule.

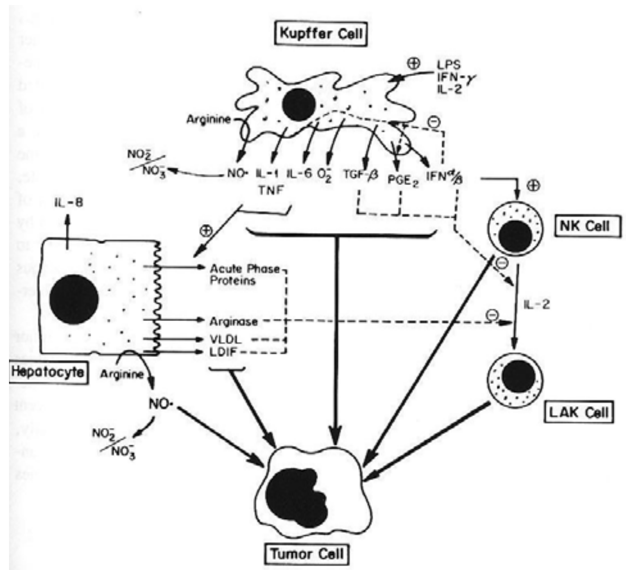

Figure 5. Liver cell-cell interactions.in hepatic lobule (Billiar and Curran 1992)

Knowledgebase development & text-mining. Open source tools will be used to build the KB (Protégé, Noy et. al. 2000), which will be coupled with an OWL-compliant (Antoniou and Harmelen 2004) database-backend for storage (e.g. Sesame), and an open-source reasoning engine to support queries. The knowledgebase will be bootstrapped by manually curating facts on molecules, cells, lobule structure and their interactions related to normal physiology and its perturbation by NRs leading to non-genotoxic cancer. The initial facts will extended using public domain information sources including NCBI Genome, Gene, Interaction, Protein; GeneOntology (Harris et. al. 2004); SwissProt (Boeckmann et. al. 2003); KEGG (Kanehisa et. al. 2006); MetaCyc (Caspi et. al. 2006); and Reactome (Vastrik et. al. 2007). Open source text-mining tools will be evaluated to semi-automate the identification of relevant entities and the extraction of facts about their relationships from PubMed abstracts or full-text articles (Palakal el. al. 2002; Hu el. al. 2004; Muller el. al. 2004; Doms and Schroeder 2005; Wilbur el. al. 2006; Yuryev el. al. 2006). This effort will be closely coordinated with the Virtual Embryo project, which has similar text-mining needs.

Multiscale Modeling and Simulation. Information about the complex interactions between molecular, cellular and tissue level processes organized in the KB will be used to develop dynamic models of chemical-induced response. First, mechanistic models describing early molecular interactions of environmental chemicals with nuclear receptors (NRs) namely, CAR, PXR and PPAR-α will be developed. We plan to use Probabilistic Boolean Networks (Kauffman 1993; Shmulevich et. al. 2002) initially to understand the overall dynamics of NR-mediated signaling and interspecies differences. If sufficient quantitative data on molecular entities become available, traditional continuous deterministic models will also be explored. Second the intracellular molecular response modules leading to changes in cell state, namely proliferation and apoptosis, will be modeled. Since changes in cell state are being represented categorically, they will be simulated using discrete stochastic simulation methods. These models will advance the two-stage clonal growth models of cancer (Conolly and Andersen 1997). In addition, they will include intercellular descriptions of paracrine signaling between parenchymal and non-parenchymal cells, which are known to play a role in liver injury (Rusyn 1998; Roberts 2007; Reeves 1996; Krutovskikh et. al. 1995). The third biological scale integrates these models into a spatial model of the hepatic lobule. At this scale, the response of cellular networks is simulated in the context of portal to centrilobular blood flow, represented as a gradient of of nutrients and xenbiotics. The spatial model of the hepatic lobule will be initially developed using an agent-based modeling (ABM) approach (Axelrod, 1997; Epstein and Axtell, 1996; Athale *et. al.*. 2005) in which cells will be modeled as autonomous agents. ABM is a useful formalism because it can (i) predict emergence of chronic tissue injury due to simple interactions between a few cell types; (ii) describe the lobular architecture naturally using descriptions of intercellular communication; (iii) encapsulate adaptive and complex cellular responses to xenobiotic and nutrient inputs; and (iv) directly utilize information in the Liver KB about tissue components and their relationships. If resources are available, we also propose to integrate this into a pharmacokinetic model to estimate dosimetry and subsequent damage. Performance metrics will be developed for evaluating the accuracy of the Virtual Liver modules for predicting dose-dependent injury.

5. What other components of EPA or outside organizations are involved?

Relationship with existing EPA efforts. The Virtual Liver Project will be synergistic with the following projects / individuals: ToxCast™ (Dix *et. al.* 2007); The Virtual Embryo; ToxRefDB (Martin *et. al.* 2007); ACToR (Judson *et. al.* 2007); metabolomics (Tim Collette, NERL); exposure modeling (Miles Okino, NERL); *in vivo / in vitro* experiments and modeling endocrine disruption by NR-activators leading to thyroid toxicity (Mike DeVito, NHEERL); domain expert providing input on liver histopathology (Doug Wolf, NHEERL); risk assessment (Rob Dewoskin and Paul Schlosser, NCEA); transript profiling (Chris Corton, NHEERL); systems biology (Stephen Edwards, NHEERL); steering committee (Julian Preston, NHEERL); physical simulation and modeling (Richard Spencer, Lockheed Martin); knowledgebase development (Lynn Meredith, Lockheed Martin). We are also initiating dialogue with the EPA program offices to identify key stakeholders in the project.

Relationship with government agencies. The Virtual Liver team is communicating with the NIEHS (David Balshaw) to establish links with hepatotoxicity-related intramural research efforts, the NIBIB multiscale simulation initiative and with the joint European Commission-US Task Force on Biotechnology to identify areas of common research sinterest on virtual tissues with Europe. In order to facilitate discussions and engage experts in the field, we are planning to hold a workshop in Fall 2008.

Relationship with non-profit / academic institutions. The Virtual Liver team is collaborating with the Hamner Institute on chronic liver toxicity experiments, genomics and co-culture *in vitro* systems; and are in discussions with EPA funded STAR centers (UNC, CH and UMDNJ) on toxicology, systems modeling and text-mining; Center for Computational Pharmacology, University of Colorado on knowledgebases and text-mining; National Institute of Biomedical Ontologies for ontology development. Initial discussions have also taken place with members of the SBML team while communication with the Physiome project is underway to ensure that models developed by the project will be freely available using existing standards.

1. How is data management being achieved?

All empirical data including molecular, cellular and phenotypic assays will be managed in ACToR (Judson *et. al.* 2007). Information about mechanisms or the mode of action will be managed in the Liver KB , which will be freely made available in OWL (Web Ontology Language) and through custom web-based interfaces for searching and browsing; and, biological models will be made freely available using existing standards CellML (Nickerson, 2005) or SBML.

7. What are the measures of success?

<u>Short-term:</u>

- Organizational: Identification of key stakeholders / risk assessors and domain experts to serve as curators/reviewers for KB; recruitment of key participants
- Deployment of Liver KB representing key molecular and cellular pathways for NR-mediated non-genotoxic cancer (between rodents and humans)

- *In vitro* data generation for use in molecular and cellular models
- Initial dynamic models for (a) NR-mediated regulation of xenobiotic metabolism and (b) hepatocytes and Kupffer cell interactions
- Peer-reviewed publication of KB and predictive models in computational / toxicology journals
- Outreach: Educate internal/external scientific community on the roles/needs for system modeling in environmental toxicology

Long-term:

- Expansion of Liver KB to include additional modes of chronic liver injury
- Multiscale liver tissue model for predicting chronic injury
- *in vivo* data for system evaluation / refinement
- Publication of results & outreach
- Impact on-going risk assessment

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